PHARMACOKINETICS OF INTRAMUSCULAR MORPHINE IN THE HORSE

by

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Abstract

Pharmacokinetics of Intramuscular Morphine in the Horse

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Objective - To determine the pharmacokinetics of morphine after intramuscular administration in a clinical population of horses

Design – Prospective, clinical study

Animals – Pilot study included 2 normal horses and the clinical study included 75 horses

Procedures – Morphine was administered at 0.1mg/kg, IM and 2-3 blood samples were obtained from each horse at various times from 0-9 hours after administration. Plasma morphine concentrations were measured using liquid chromatography and mass spectrometry.

Results – Data was analyzed using a naïve pooled pharmacokinetic model. The half-life for the elimination phase was approximately 1.5 hours, the volume of distribution (per bioavailability) was approximately 4.5 L/kg and the clearance (per bioavailability) was approximately 35 mL/kg/min. The peak plasma concentration was 21.6 ng/mL and occurred approximately 4 minutes after administration. Plasma concentrations of morphine were below the limit of quantification by 7 hours in 74 horses.

Conclusions and Clinical Relevance – The relatively short half-life of morphine indicates the need for frequent dosing to maintain targeted plasma concentrations. Adverse effects were uncommon in this study and morphine was well tolerated at a dose of 0.1 mg/kg, IM. Morphine may be a useful adjunctive therapy in painful horses, but the variable plasma concentrations suggest the dose and dosing interval may need to be adjusted to the individual patient's response.

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Chapter 1 - Introduction

The use of analgesics in the horse has been evolving over the past few decades. As veterinarians and horse owners have become more conscious of the importance of pain management, there has been a push toward using multimodal analgesia. Morphine is a μ-opioid agonist that was routinely used in equine practice as a pre-anesthetic and analgesic. However, the use of morphine in equine practice has waned due to the potential for adverse effects. Gastrointestinal stasis [1], and CNS excitation [2] are documented adverse effects of morphine administered at high doses. Newer classes of drugs, such as alpha-2 agonists and agonist/antagonist opioids, were associated with fewer adverse effects and began to take the place of morphine in routine practice. As the importance of analgesia has become more readily accepted, the use of NSAIDs has become a staple in equine practice. However, the use of these medications is not without consequence. Complications of NSAID administration include right dorsal colitis, gastric ulceration, and renal failure. In spite of the adverse effects, NSAIDs are widely used due to their anti-inflammatory and analgesic properties. However, there are some conditions, including laminitis, infected synovial structures, and fractures, where the analgesia achieved by using NSAIDs alone is inadequate. Multimodal analgesia, such as the addition of an opioid, can be beneficial when NSAID administration alone is not suffieicient.

Most pharmacokinetic studies use a small number of healthy horses, typically 4-8, and frequent plasma sampling to determine the absorption, distribution, metabolism, and excretion of the drug in each individual animal in a homogenous group of animals. The mean, median, and/or range of the pharmacokinetics parameters are reported. These studies, termed standard two-stage studies, are useful for predicting the average parameters in a well defined homogenous population. However, a small, homogenous group of healthy animals may not be representive of the clinical population of horses that might benefit from morphine as an analgesic.

Several other methods for assessing the pharmacokinetics of a drug are available including population pharmacokinetics and naïve pooled pharmacokinetics. In contrast to standard two-stage pharmacokinetic studies, population and naïve pooled pharmacokinetic studies may use large numbers of animals, often including the target clinical population, and do not require intensive sampling from individual patients. Population pharmacokinetics includes a

large, more diverse group and allows biologic variability to be evaluated. Factors affecting the variability in a veterinary population may include age, breed, body weight, concurrent medications, and health status. [3, 4] Population pharmacokinetics allows identification of patient traits which may alter the pharmacokinetics of a drug. However, one of the disadvantages of population pharmacokinetics is that fitting a model to the data can be very difficult with some data sets.

Naïve pooled pharmacokinetic studies are often used to minimize sampling from any individual patient and to allow for the study of a group of patients. They include large numbers of individuals and can be performed in the target clinical population. In contrast to population pharmacokinetics, a single model is fit to pooled data from all of the animals. Therefore individual factors such as age, breed, and weight cannot be assessed for effects on pharmacokinetic parameters. However, naïve pooled pharmacokinetic studies typically do not require the specialized software needed for population pharmacokinetics, and a single model is fit to all of the data making the modeling less complex.

Even though morphine has been used for many years in horses, the pharmacokinetic parameters are not well described. The dose and dosing frequency of IM morphine have been empirical, based on the practitioner's experience, and on the horse's degree of discomfort. Due to the adverse effects that can occur with excessive administration, it is imperative that a proper dose and dosing frequency be clarified to minimize these effects. The first step to describing an appropriate dose is to determine the pharmacokinetic parameters of the drug using the desired route of administration. The purpose of this study is to determine the pharmacokinetics of morphine after intramuscular administration in a clinical population of horses. This information was obtained from clinical cases in order to assess the variability in the plasma concentrations of morphine in a population of patients instead of healthy research animals.

Chapter 2 - Materials and Methods

The pilot study and clinical phase of this project were approved by the Institutional Animal Care and Use Committee and were carried out in accordance with their guidelines. All clients were informed of the nature of this study and consented to the use of their horse prior to inclusion.

Pilot Study – Two healthy Quarter Horses were used for this study. The left jugular vein was catheterized to allow for serial blood sample collection. The horses were weighed and morphine was administered intra-muscularly at a dose of 0.1 mg/kg in the left side of the neck. Heparinized whole blood samples were obtained at 5, 10, 15, 30, and 45 minutes, and 1, 2, 4, 6, 8, 10, and 12 hours after morphine administration. Paired sets of blood samples were obtained from one horse. Physical exams were performed at the time of morphine administration and every 2 hours for twelve hours to monitor for any potential adverse reactions. This exam included heart and respiratory rate, rectal temperature, borborygmi, evaluation of sedation or excitation, number of defecations, and monitoring the injection site.

The samples were centrifuged for 5 minutes and the plasma was separated and frozen within 2 hours of sample collection. The duplicate blood samples that were obtained from one horse were batched, stored on ice and centrifuged at the end of the day (ranging from 1 to over 12 hours after collection) to determine if sample handling would affect results. All plasma samples were frozen and stored in a -70^{o} C freezer prior to analysis. Morphine concentrations were determined by liquid chromatography and mass spectrometry analysis to ensure that this study design would measure morphine plasma concentrations in the clinical cases and to determine the LOQ.

Clinical Phase – Clinical cases presenting to the Kansas State University Veterinary Medical Teaching Hospital from December 2010 to June 2011 were used in the study. Horses were included if morphine was administered as a perioperative analgesic or for treatment of a painful condition. All horses continued treatment for their disease process as deemed necessary by the supervising clinician. Horses were weighed and administered intramuscular morphine (0.1 mg/kg) in the neck at time 0. The side of the neck that was used was chosen depending on where other medications were administered previously to that animal. Two or three blood

samples, 7 ml each, were collected at variable times after morphine administration to approximately 9 hours. The time of sampling was recorded to the minute and the sampling times were distributed throughout the 9 hours to allow for an even distribution of samples. The blood samples were centrifuged up to eight hours after collection and the plasma was separated and stored in a -70^{o}C freezer until analysis. Physical exams were performed at the time of morphine administration and every two hours for a total of eight hours, as well as at the time of blood collection. These physical exams included monitoring the same variables as listed above for the pilot study. Since morphine concentrations were not reliably detectable past 8 hours in the pilot horses, monitoring physical exam parameters was discontinued at 8 hours in the clinical population. Observations were not obtained if the horse was in surgery, recovering from surgery, or discharged from the hospital at the time of the check point. Concurrent medications, including anesthetic agents and intravenous fluids, were recorded for each patient along with breed, age, weight, sex, and diagnosis.

Plasma Drug Analysis – Liquid chromatography and mass spectrometry were used to measure the morphine concentrations in the plasma samples. Samples were thawed on a 40^{o}C heat block and centrifuged at 5,000 rpm for 5 min. Hydromorphone D3[a] (0.1 mL, 100 ng/mL), the internal standard, and borate buffer (1 mL, 0.1M, pH 9.2) were added to 1 mL of plasma and mixed. Bond Elut C18 solid phase extraction cartridges[b] were attached to a solid phase extraction manifold and the cartridges were conditioned with 1 mL methanol and then 1 mL of de-ionized water. The plasma, hydromorphone, and buffer mixture were run through the conditioned cartridges attached to the solid phase extraction manifold and then rinsed with 1 mL of de-ionized water. Then, 1mL of methanol was added to cause elution of the sample from the cartridge. The methanol was evaporated to dryness at 40^{o}C under an air stream for 30 minutes. The sample was then re-suspended using 0.2 mL of 50% methanol and mixed thoroughly. The sample was centrifuged at 15,000 rpm for 5 min and the supernatant was removed and placed in injection vials. Plasma morphine standards were processed in an identical manner as the samples at least twice daily to ensure consistency of the assay. The interday accuracy of the assay determined on replicates of 5 at each of the following concentrations (2.5, 10, and 50 ng/mL) were 102, 98 and 99%, respectively, of the actual concentration. The interday coefficients of variation determined on replicates of 5 at each of the following concentrations (2.5, 10, and 50 ng/mL) were 9, 9, and 7%, respectively. The analytical LOQ was 2.5 ng/mL defined as the

lowest concentration of the standard curve with measured concentrations within 15% of the actual concentration.

Pharmacokinetic Analysis – Only plasma concentrations that were above the LOQ were included in the pharmacokinetic analysis. A one compartment open model with first order input and output with no lag was used for pharmacokinetic analysis of the pilot horses and naïve pooled pharmacokinetic analysis. Population pharmacokinetic modeling was assessed with a software program using a nonlinear mixed-effects model[c]. One compartment and two compartment models with first order input and output with and without lag were assessed. The primary pharmacokinetic parameters estimated were the V_d, absorption rate constant, and elimination rate constant. The secondary pharmacokinetic parameters included the apparent absorption half-life, apparent elimination $t_{1/2}$, area under the curve, maximum plasma concentration, and time to the maximum plasma concentration.

Statistical Analysis – A Friedman repeated measures ANOVA on ranks test was used to analyze the heart rate and respiratory rate data with the level of significance set at $P<0.05$. A comparison between the duplicate samples was made by calculating the difference between the samples centrifuged within 2 hours and those that were batched for later centrifugation. Then, a linear regression model was used to determine a relationship between the samples centrifuged within 2 hours of collection and those centrifuged up to 12 hours after collection. Statistical software was used for data analysis[d].

Chapter 3 - Results

Pilot Study – Physical exam parameters remained constant throughout the 12 hour time period in the two pilot study horses. These horses had normal gastrointestinal motility and did not exhibit any signs of colic. Horse 1 defecated between 2-4 hours and again between 4-6 hours and horse 2 defecated between 4-6 hours and again between 8-10 hours. No adverse effects were observed during the pilot study.

Morphine concentrations in plasma peaked (CMAX) in the pilot horses at 15 min and 30 min with concentrations of 36.6 and 29.9 ng/mL respectively. Concentrations were above the LOQ (2.5 ng/mL) for 8 hours in horse 1 and 6 hours in horse 2. For the samples that were duplicated in one horse, concentrations were compared between the two samples and the percent difference between the values at the same time points was determined. Using a linear regression model with a weighting factor of $1/y^2$, the R^2=0.9910 ($P<0.0001$) indicated a strong linear relationship between the samples centrifuged at different times (1-6 hours after collection) suggesting the samples were stable after collection.

Clinical Phase – Seventy five horses were included in the clinical phase of this study. One horse was included twice because it received morphine on two separate visits to the clinic. Quarter Horses were over represented in this study with 52/75 horses. Other breeds that were represented included Warmblood, Paint, Draft breed, Tennessee Walking Horse, Thoroughbred, Arabian, Mustang, Rocky Mountain Spotted Horse, Pony of America and mixed breeds. There were 18 mares, 22 geldings and 35 stallions included. The average age was 6.2 years, with a range from 7 months – 31 years. The weights of these animals ranged from 204 – 896 kg with a mean of 415 kg.

Of the 75 horses, there were a total of 17 orthopedic cases. Six of these cases did not have surgery and these cases included laminitis, foot abscess, end stage osteoarthritis, a laceration with an open joint, and fractured sacral and caudal vertebrae. There were eleven surgical orthopedic cases, including arthroscopy, pastern arthrodesis, fracture repair, apical sesamoid fracture removal, superior and inferior check ligament desmotomy and a sequestrum removal from a splint bone. Thirty-three of the cases were castrations, with four of those being cryptorchid castrations. There were 15 cases that had a soft tissue surgery other than a

castration, which included upper respiratory surgery, post-operative colic, rectovaginal tear, palmar digital neurectomy, neuroma removal, abdominal hernia repair and an esophageal diverticulum repair. Three horses had oral or facial conditions such as incisor removal, a mandibular fracture and a frontonasal sinus flap for ventral conchal sinusitis. Four horses had ocular procedures including the removal of the third eyelid, ocular squamous cell carcinoma removal and an enucleation after a ruptured corneal ulcer. The final category included two horses with lacerations.

The respiratory rate was unaffected by morphine administration and had median values ranging from 40-44 beats per minute and 18-24 breaths per minute respectively (Table 1). There was no difference in respiratory rate at any time point (P=0.742). The heart rate at 8 hours (median 40 bpm) was different than the heart rate at 4 hours (median 44 bpm) (P=0.028). Fecal output was recorded every two hours for each horse (Figure 1). There were 22 horses that left the clinic before 8 hours, so some horses may have defecated during the eight hour period that were not recorded. There were 17 horses in the study that did not undergo general anesthesia. Of these cases, there were two that defecated within the first 2 hours and another three defecated from 2-4 hours, whereas feces were found in four horse's stalls between 4-6 hours. The rest of the horses either did not defecate by eight hours (n=2) or they were discharged before 8 hours (n=6). Mild excitation, defined as restlessness in the stall, was observed in one horse 4 hours after morphine administration. This patient was a colt presenting for castration that was not halter broken and had never been stall confined. Sedation was observed in 31 horses; however, in most cases, sedation was observed either immediately after return to the stall from anesthesia or after the administration of an alpha-2 agonist (xylazine or detomidine) or a phenothiazine tranquilizer (acepromazine). There were two horses that appeared sedate and were not administered other medications. Mild sedation, defined as a lack of interest in their surroundings, was observed in one horse at 6 and 8 hours and the other horse at 4 and 6 hours after morphine administration.

Two horses had injection site reactions. One horse had a 2.5 cm focal, circumscribed swelling at the injection site that was non-painful and appeared around 4 hours after administration. This condition persisted to the end of the eight hour observation period, but was normal the next day. The other horse had a 1.5cm, non-painful swelling that was noted 4 hours

after the injection as well. By eight hours, the mass was half the size it was earlier, was still non-painful, and had completely resolved by the next morning.

Morphine was detectable (>2.5 ng/mL) in all 74 samples taken less than 3 hours after morphine administration. There were 49/51 samples taken from 3-5 hours after injection with concentrations >2.5 ng/mL. From 5-7 hours, only 13 out of 48 samples were above 2.5 ng/mL. Only one sample had a morphine concentration >2.5 ng/mL after seven hours.

An attempt was made to fit the data to a population pharmacokinetic model, but this could not be accomplished satisfactorily. Therefore, a naïve pooled population analysis method was utilized to determine the pharmacokinetic parameters of morphine (Table 2). In both pilot horses and in the clinical population, IM morphine had a rapid absorption phase followed by a slower elimination phase (Figure 2). The two pilot horses had higher peak concentrations (28.2 ng/mL and 33.5 ng/mL) compared to the naïve pooled analysis of the clinical population (21.6 ng/mL). While the data for the individual pilot study horses showed higher concentrations of drug than the naïve pooled pharmacokinetic model for the clinical population, the pilot horses still fit within the range of data for the clinical cases. The absorption rate constant for the naïve pooled analysis of the clinical horses was 79.76 h^{-1} and was 8.21 and 17.68 h^{-1} for each of the pilot horses. The apparent half-life of the absorption phase was 5 and 2.35 min the pilot horses and was only 30 seconds in the naïve pooled analysis of clinical horses. Maximal concentrations of morphine peaked at around 24 and 13 min in the pilot horses and the peak concentrations occurred in the naïve pooled analysis of the clinical horses at approximately 4 minutes after morphine was administered IM. The apparent elimination $t_{1/2}$ was 1.48 hr in the naïve pooled analysis and 1.76 hr in the pilot horses.

Since no intravenous model was included in our study, bioavailability cannot be calculated. Therefore, V_d and Cl are both calculated relative to F and are not absolute. These parameters can be used for other calculations using intramuscular morphine, but do not imply 100% bioavailability of the drug. The V_d/F of the pilot horses was 3.07 and 2.72 L/kg and 4.49 L/kg for the naïve pooled data. The Cl/F for the clinical population is 34.9 mL/kg/min and is 18.5 and 19.6 mL/min/kg in the pilot study.

Figures and Tables

Figure 3.1 Time to first defecation

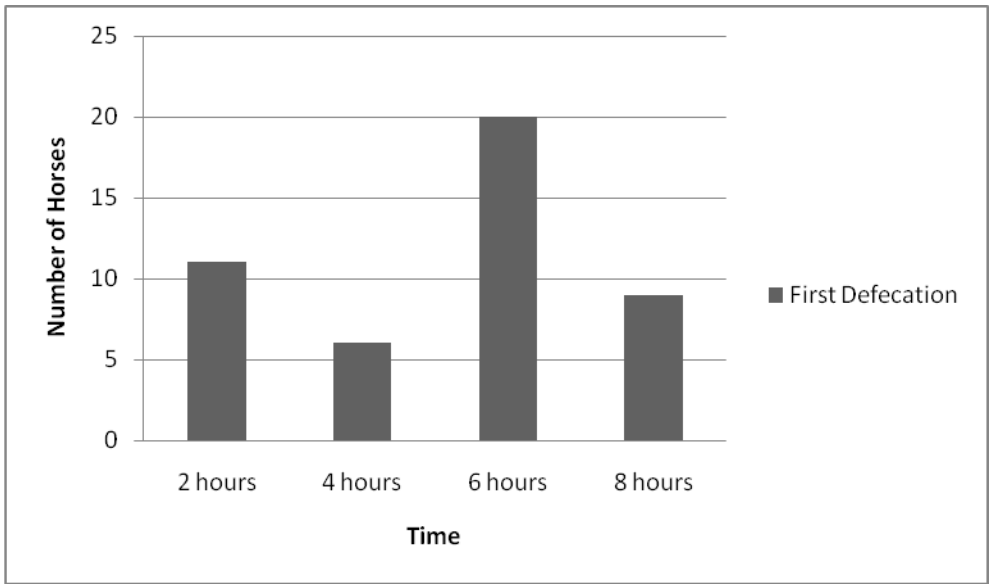
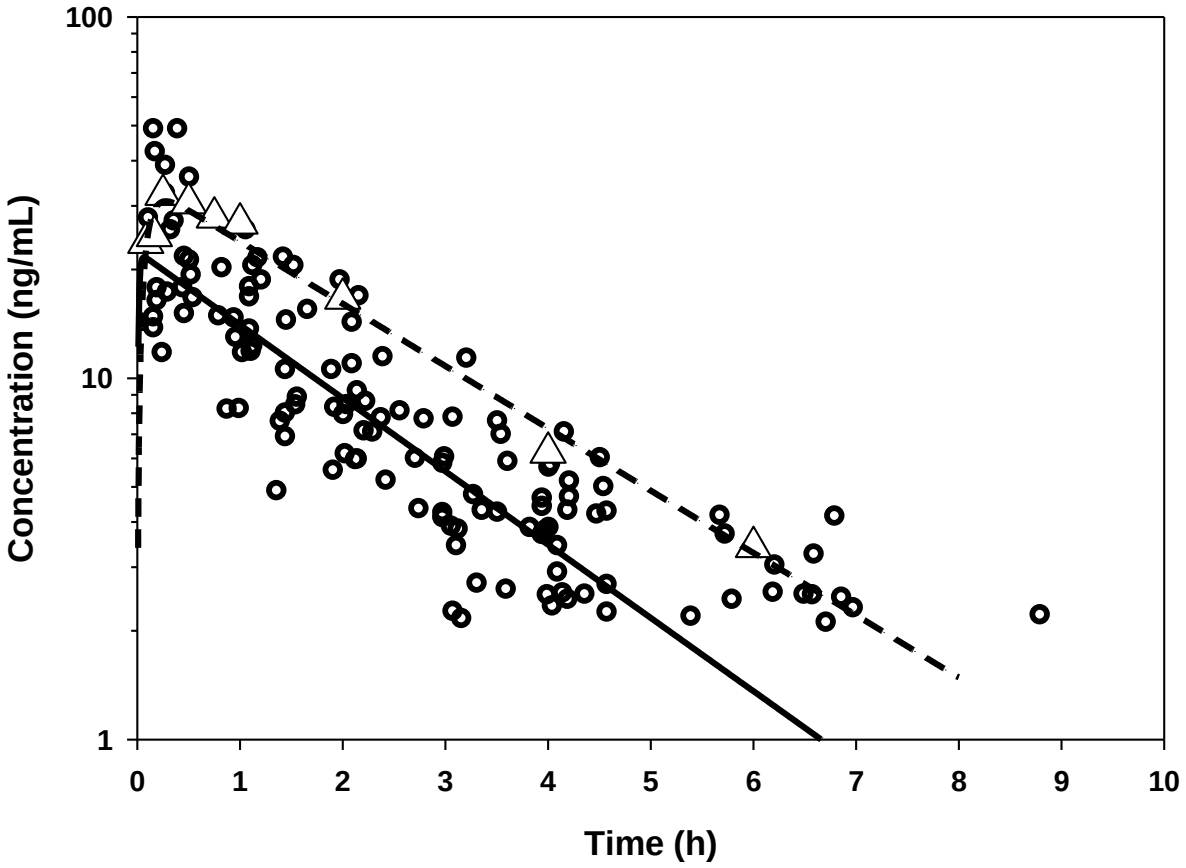


Figure 3.2 Semi-log plot of the plasma profile of IM morphine (0.1 mg/kg) in horses.



Solid line = naïve pooled predicted plasma profile. Dashed line = pilot predicted plasma profile. Open circle = plasma concentrations from naïve pooled analysis. Open triangle = mean plasma concentration pilot horses.

Table 3.1 Heart and Respiratory Rate (per minute)

	Respiratory rate				Heart Rate		
Time	Min	Max	Median		Min	Max	Median
0	12	66	20		28	80	44
2	12	100	24		28	80	44
4	12	60	18		32	72	44
6	8	72	18		28	64	43
8	12	44	20		28	64	40

Table 3.2 Pharmacokinetics following intramuscular morphine (0.1 mg/kg) in horses.

Parameter	Units	Pilot Horse Mean	Pilot Horse Range	Naïve pooled
Ka	h^{-1}	12.95	8.21 - 17.68	79.76
$t_{1/2}$ abs	h	0.0618	0.0392 - 0.0844	0.00869
Kel	h^{-1}	0.397	0.361- 0.432	0.467
$t_{1/2}$	h	1.76	1.6 - 1.96	1.48
V_d/F	L/kg	2.9	2.72 - 3.07	4.49
AUC	h*ng/mL	87.7	85 - 90.3	47.74
Cl/F	mL/min/kg	19.05	18.5 - 19.6	34.9
CMAX	ng/mL	30.85	28.2 - 33.5	21.6
TMAX	h	0.31	0.22 - 0.4	0.065

Ka=Absorption rate constant, $t_{1/2}$ abs= Absorption half-life, Kel=Elimination rate constant, $t_{1/2}$ = Terminal half-life, V_d/F=Volume of distribution per bioavailability, AUC=Area under the curve, Cl/F=Clearance per bioavailability, CMAX= Maximum plasma concentration, TMAX=Time at CMAX

Chapter 4 - Discussion

Morphine has been used as an analgesic in horses for years, but has dose related adverse effects. Interestingly, there are a number of studies looking at variable doses after both intravenous and intramuscular administration and their clinical effects in the horse [1, 5-8], but the pharmacokinetic parameters for intramuscular morphine in the horse are not reported. The pharmacokinetic parameters of morphine (0.1 mg/kg, IV) in horses resulted in a terminal half-life of 1.6 hours and mean plasma concentrations were below 10 ng/mL by 4 hours and below 5 ng/mL by 6 hours. A three compartment model was used to describe the pharmacokinetics of IV morphine in the previous study [9]. The pharmacokinetics of intravenous morphine (0.25 mg/kg) during isoflurane anesthesia in horses included a terminal half-life of 0.7 h, V_d =1.2 L/kg and Cl=40 mL/min/kg. In the same study, the $t_{½}$=1h when a dose of 2 mg/kg IV was administered. These authors postulated that elimination $t_{½}$ of IV morphine is dose dependant[10].

Administration of a medication into a compartment other than the central venous compartment can alter the plasma profile due to absorption, and subsequently the apparent elimination $t_{½}$ of extravascular morphine may be substantially different for different routes of administration. Additionally, poor absorption from extravascular routes may result in low and sub-therapeutic concentrations. Therefore, it is important to describe the pharmacokinetic parameters of IM morphine in horses to assess whether it could be a clinically useful route of administration. The pharmacokinetic parameters of morphine in the clinical horses included an apparent elimination $t_{1/2}$ of 1.5 h, a V_d/F of 4.49 L/kg, and a Cl/F of 34.9 mL/min/kg.

A one compartment open model with first order input and output with no lag was used for pharmacokinetic analysis of the pilot horses and naïve pooled pharmacokinetic analysis. The model was chosen based on visual inspection of the data and the model that best fit the pilot data. Although the plasma concentrations below the LOQ were not quantifiable, morphine was still likely present, just below 2.5 ng/mL. Therefore, it is anticipated that if the actual concentrations in the samples below the LOQ were able to be quantified with a more sensitive assay, they would have resulted in uniform residuals on the terminal portion of the curve suggesting a one compartment model is the most appropriate model for the naïve pooled dataset. However, it is

possible that a two compartment model could best describe this dataset if our assay was sensitive enough to quantify concentrations lower than the LOQ.

The plasma morphine concentrations of the pilot horses were within the range of the patient population, despite the numerical differences in some of the pharmacokinetic parameters. Figure 2 demonstrates the large variability of plasma morphine concentration after IM administration to a large population of animals. It is important to recognize the variability of IM morphine in horses and routine clinical monitoring of these patients is necessary to maximize drug efficacy and minimize adverse effects and that a single dose rate will not adequately treat all patients.

Some differences in the pharmacokinetic parameters were present between the naïve pooled horses and pilot horses. Sampling during the pilot study included intense sampling during the absorption and elimination phases, while the naïve pooled analysis had sampling primarily during the elimination phase. This was done intentionally to completely capture the elimination phase, as this is more clinically important for dosing interval than the absorption. However, our data may incompletely represent the absorption phase, thus biasing the absorption rate and Tmax in the naive pooled analysis, which is a limitation of this study. The lack of samples in the first 15 minutes may also have contributed to the inability to fit a population pharmacokinetic model to the data.

Dose ranges reported for morphine in horses varies anywhere from 0.02 – 2.4 mg/kg IV, in both experimental and clinical cases. [8, 11] Higher doses have been associated with more adverse effects in horses, which include decreased gastrointestinal motility and CNS excitation. At a dose of 1 mg/kg, morphine slowed the passage of feces in the gastrointestinal tract of horses.[1] Boscan et al. reported a significant decrease in fecal moisture content and an increase in GI transit time after repeated doses every 12 hours of 0.5mg/kg of IV morphine.[7] The treated horses had a 22 hour delay in passing barium impregnated spheres compared to the control group. Increased CNS stimulation occurred for 2-4 hours after the administration of 0.5 mg/kg IV morphine in three of the five horses in the study. In their pilot study, giving 3 treatments of 1mg/kg of IV morphine caused severe colic signs in one of two horses. An IM dose of 0.66 mg/kg also caused restlessness in 7/8 ponies, which started 1 hour after administration and lasted for about 3 hours.[5] When administered at a dose of 2.4 mg/kg, horses became ataxic and unaware of their surroundings. [11] In this study, a lower dose (0.1mg/kg) was administered and

there were minimal effects on heart rate, degree of excitation, and no signs of colic observed in the study population. A retrospective study was performed which evaluated morphine-related adverse events in horses that received a dose of 100-170 μg/kg (0.1-0.17 mg/kg). These authors concluded that the use of morphine did not increase the risk of intra-operative or post-operative complications.[2] This dose has been used clinically as an analgesic at other institutions [7] and is the dose that is used intramuscularly at Kansas State University.

Morphine administered IM to the clinical horses was only consistently detectable with our assay for three hours. After that time, in some horses, the morphine plasma concentration was less than 2.5ng/mL. Due to the lack of detectable morphine concentrations during the later time points in the study, it is likely that frequent dosing is necessary in the clinical patient to provide consistent analgesia.

The efficacy of this dose intramuscularly was beyond the scope of this study. Pharmacodynamic studies correlating pharmacokinetic information with the analgesic response have not been reported in the horse and would be a potential area for further research. Pharmacodynamic studies have been performed in humans, and while this information cannot be extrapolated directly to horses, it does provide some general guidelines to allow equine practitioners to make educated decisions about dosing recommendations. In a prospective study of human cancer patients with chronic pain, there was substantial individual variability seen in the serum concentrations of morphine needed for analgesia. The range of effective plasma concentrations was 30-120 nmol/L (8.58-34 ng/mL) for the 25-75th percentiles.[12] When patient controlled analgesia was examined in the immediate post operative period, there was also high individual variability. The mean minimum effective plasma concentration and standard deviation was 16 ± 9 ng/mL.[13] If horses follow a similar pattern and 16 ng/mL is used as an approximate mean minimum analgesic concentration, only 18 of the 27 samples taken between 0-1 hours had morphine concentrations above this level. From 1-2 hours, there were 25 samples taken and only 9 samples exceeded 16 ng/mL. If analgesic plasma concentrations are well correlated to human findings, then a 0.1mg/kg dose of IM morphine may not consistently provide analgesia to most of the population. While this model cannot predict the safety of other doses, it can be utilized to predict the plasma concentrations of morphine after the IM administration of other doses. According to our model, if this population of horses was administered 0.2 mg/kg intramuscular morphine and the plasma concentrations are proportional

to the dose, all of the 27 samples taken between 0-1 hours would have been over 16 ng/mL. Twenty of the 25 samples from 1-2 hours would have been over 16 ng/ml with this increased dose. From 2-3 hours, 8 of the 22 horses sampled during this time would have been over this targeted concentration. The effect of changes in dose can be extrapolated with this model; however, as shown in previous studies [1, 7], increasing doses of morphine may increase the number of adverse effects. Therefore studies should be conducted assessing the pharmacokinetic parameters and adverse effects of higher morphine doses, such as 0.2 mg/kg. It is apparent from Figure 2 that marked variability in morphine plasma concentrations occurred in clinical patients. This model does suggest that higher doses will be needed to provide targeted plasma morphine concentrations to a larger percentage of horses and the plasma concentrations will last for longer than the dose used in this study. Clinical judgment is required in determining the appropriate dose to maximize analgesia and minimize adverse effects.

Morphine consumption from patient controlled analgesia was measured in the previously mentioned human studies with morphine as the only analgesic administered. While the administration of one analgesic is effective, the use of more than one class of analgesics can also be beneficial. Multimodal analgesia combines analgesics with different mechanisms of action in an attempt to enhance analgesia and minimize the adverse effects of the individual medication.[14, 15] A systematic review of 60 human studies concluded that the morphine required for analgesia was significantly reduced by the concurrent administration of an NSAID. There was a significant decrease in post-operative nausea and vomiting, which are adverse effects of morphine in people, in the group that received an NSAID and morphine.[16] There is clear evidence that multimodal analgesia is effective in human medicine and case reports suggest the same is true in horses.[14] In this study, almost every horse that received morphine was also administered either phenylbutazone or flunixin meglumine. The effects of concurrent administration of an NSAID on morphine analgesic effects have not been reported for horses. It is likely that combination therapy in horses will decrease the plasma concentration of morphine needed for analgesia, but further study is needed to determine effective concentrations of morphine when used alone or in combination.

One limitation of our study was that the observer assessing sedation and excitation scores was not blinded and there could be bias in these observations. Another limitation is that it is difficult to draw any meaningful conclusions about fecal output, because most horses also

underwent general anesthesia and had feed withheld for 6-8 hours before surgery. Even without opioid administration, general anesthesia has been shown to slow gastrointestinal motility.[17] Since most of the horses were passing feces, it seems reasonable to conclude that if morphine administered at this dose inhibits motility, the effect is transient. However it is important to note that the effects of higher or multiple doses on GI motility were not evaluated.

Most cases (59/76) had anesthesia and a surgical procedure the same day that morphine was administered. A comparison of the pharmacokinetics of morphine with and without concurrent general anesthesia has not been reported. Similarly the effects of health status, breed, or gender have not been reported in horses. Therefore numerous factors may have impacted the pharmacokinetic parameters of morphine in this study. This data was obtained in clinical patients and therefore should be more representative of the pharmacokinetics of the target population than data obtained in a study of healthy horses.

An injection site reaction was noted in two of the horses. Both horses had a raised, non-painful lump at the injection site that resolved without therapy. Causes of the swelling may have included a hematoma, seroma, or allergic reaction. However, since it was self-limiting and transient, there were no further diagnostics performed to determine the nature of the swelling. The authors are unaware of any other reports describing an injection site reaction after intravenous or intramuscular administration. A statistically significant difference in heart rate of patients was seen at 4 and 8 hours post morphine administration. However, the median heart rate at 4 hours was 44 bpm and at 8 hours was 40 bpm, which are both within the normal reference range. Therefore, this statistical difference (P=0.028) does not appear to have any clinical relevance.

Time from sample collection to centrifugation and freezing was a variable during the clinical study, but data collected in the pilot study indicated that time from collection to centrifugation of the sample resulted in minimal differences in the measured plasma concentration. This was important due to the nature of this study in which clinical cases were sampled, as it can be challenging to obtain multiple blood samples from each patient and centrifuge them immediately after sampling. By comparing duplicate samples from one pilot horse, it was concluded that separating the plasma up to 6 hours after collection had minimal effects on the amount of morphine that was detected in the plasma.

This study provides valuable information about the pharmacokinetic parameters and tolerability of intramuscular morphine in a population of clinical patients. At a dose of 0.1 mg/kg, only about two thirds of the samples taken during the first hour reached morphine plasma concentrations equivalent to those that are efficacious in humans. While many factors can contribute to correlating pharmacokinetic and pharmacodynamic effects in the horse, this study demonstrates that this dose may be too low to provide analgesia in most clinical patients as a sole analgesic. The apparent elimination $t_{1/2}$ of intramuscular morphine was found to be relatively short ($t_{1/2}$=1.5hr), which indicates that frequent dosing is likely needed to provide consistent analgesia. While a 0.1 mg/kg dose was found to produce few adverse effects in our clinical population, this study cannot predict the safety of IM morphine at other doses or dosing intervals. Morphine may be a viable option for analgesia in painful horses, but care should be taken to monitor these horses for efficacy and adverse effects in order to use the lowest dose that is therapeutic for the individual.

References

1. Alexander F. The effect of some anti-diarrhoeal drugs on intestinal transit and faecal excretion of water and electrolytes in the horse. *Equine Vet J* 1978;10:229-234.
2. Mircica E, Clutton RE, Kyles KW, et al. Problems associated with perioperative morphine in horses: a retrospective case analysis. *Vet Anaesth Analg* 2003;30:147-155.
3. Cox SR, Liao S, Payne-Johnson M, et al. Population pharmacokinetics of mavacoxib in osteoarthritic dogs. *J Vet Pharmacol Ther* 2011;34(1):1-11.
4. Peyrou M, Doucet MY, Vrins A, et al. Population pharmacokinetics of marbofloxacin in horses: preliminary analysis. *J Vet Pharmacol Ther* 2004;27:283-288.
5. Kalpravidh M, Lumb WV, Wright M, et al. Effects of butorphanol, flunixin, levorphanol, morphine, and xylazine in ponies. *Am J Vet Res* 1984;45:217-223.
6. Phaneuf LP, Grivel ML, Ruckebusch Y. Electromyoenterography during normal gastro-intestinal activity, painful or non-painful colic and morphine analgesia, in the horse. *Can J Comp Med* 1972;36:138-144.
7. Boscan P, Van Hoogmoed LM, Farver TB, et al. Evaluation of the effects of the opioid agonist morphine on gastrointestinal tract function in horses. *Am J Vet Res* 2006;67:992-997.
8. Clark L, Clutton RE, Blissitt KJ,et al. The effects of morphine on the recovery of horses from halothane anaesthesia. *Vet Anaesth Analg* 2008;35:22-29.
9. Combie JD, Nugent TE, Tobin T. Pharmacokinetics and protein binding of morphine in horses. *Am J Vet Res* 1983;44:870-874.
10. Steffey EP, Eisele JH, Baggot JD. Interactions of morphine and isoflurane in horses. *Am J Vet Res* 2003;64:166-175.
11. Combie J, Dougherty J, Nugent E, et al. Pharmacology of Narcotic Analgesics in the Horse IV. Dose and Time Response Relationships for Behavioral-Responses to Morphine, Meperidine, Pentazocine, Anileridine, Methadone, and Hydromorphone. *J Equine Medicine Surg* 1979;3:377-385.

12. Klepstad P, Dale O, Kaasa S, et al. Influences on serum concentrations of morphine, M6G and M3G during routine clinical drug monitoring: a prospective survey in 300 adult cancer patients. *Acta Anaesthesiol Scand* 2003;47:725-731.
13. Dahlstrom B, Tamsen A, Paalzow L, Hartvig P. Patient-controlled analgesic therapy, Part IV: pharmacokinetics and analgesic plasma concentrations of morphine. *Clin Pharmacokinet* 1982;7:266-279.
14. Dutton DW, Lashnits K.J., Wegner K. Managing severe hoof pain in a horse using multimodal analgesia and a modified composite pain score. *Equine Vet Ed* 2009;21:37-43.
15. Wideman GL, Keffer M, Morris E, et al. Analgesic efficacy of a combination of hydrocodone with ibuprofen in postoperative pain. *Clin Pharmacol Ther* 1999;65:66-76.
16. Maund E, McDaid C, Rice S, et al. Paracetamol and selective and non-selective non-steroidal anti-inflammatory drugs for the reduction in morphine-related side-effects after major surgery: a systematic review. *Br J Anaesth* 2011;106:292-297.
17. Singh S, McDonell WN, Young SS, Dyson DH. Cardiopulmonary and gastrointestinal motility effects of xylazine/ketamine-induced anesthesia in horses previously treated with glycopyrrolate. *Am J Vet Res* 1996;57:1762-1770.
18. Burford JH, Corley KT. Morphine-associated pruritus after single extradural administration in a horse. *Vet Anaesth Analg* 2006;33:193-198.